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OBSERVATIONS ON THE SOMATIC CHROMOSOMES OF LAMB CELLS IN CULTURE

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OBSERVATIONS ON THE SOMATIC CHROMOSOMES OF LAMB CELLS IN CULTURE[ab]

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The number of chromosomes reported by various workers for domestic sheep (*Ovis aries*) ranges from 33 to 60. Berry (1941), Ahmed (1940) and Makino (1943) have reported a diploid number of 54 chromosomes in the spermatogonial cells of the sheep. The present observations were undertaken with the hope of analyzing the somatic chromosomes of this species with the newer technique for the spreading of chromosomes in tissue culture.

Materials and Methods

Lung tissue from a 7-day-old male lamb (*Ovis aries*) was cut into fragments of approximately 1.5 mm. side length and explanted in plasma clots on 12 x 50 mm. cover slips of No. 1 thinness. The clot consisted of equal parts of heparinized rooster plasma and extract from 8-day chick embryos. Three explants were placed on each cover slip and after the clot became firm, these were placed in test tubes. Three hours after explantation 2 ml. of Eagle's medium with 10 per cent horse serum were added to each tube. The cultures were incubated at 37° C. and rotated at 8 revolutions per hour. The fluid medium was changed on the 4th day after explantation. The technique of preparing slides for chromosome studies was based upon the method used by Hsu and Klatt (in press) with some modifications. On the 7th day the cultures were treated with a colchicine solution (0.04 ml. of a 1:10,000 dilution of colchicine in distilled water plus 2 ml. Eagle's medium) for 16 hours at 37° C. At the end of this period the cultures were treated with a hypotonic solution (sodium citrate 1.12 per cent in distilled water) for 10 minutes at room temperature. They were then fixed in Carnoy's fluid for 5 hours and stained slightly with diluted acetic orcein (0.2 gm. of orcein in 100 ml. of 50 per cent acetic acid) for ten minutes. The coverslips were extracted from the roller tubes and the explants were removed with the aid of a forceps. The coverslips carrying the cell outgrowth were inverted on to clean glass slides. They were covered with blotting paper and pressed slightly with the thumb. Finally the edge of the coverslips were sealed with Deckglasskitt nach Krönig.[e] A total of 75 explants was employed to observe the chromosomes. Observations were made with the use of a Zeiss phase contrast microscope (dark contrast 100x objective and a Zeiss 8x ocular), and a Bausch and Lomb phase contrast microscope (dark contrast 97x objective and a 12x ocular). Photomicrographs were made with a Spencer phase contrast microscope. The optical combination used consisted of a bright high contrast 97x objective and a Leitz 10x ocular.

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Observations

In the zone of outgrowth from each explant the cells were typical spindle-shaped and presumed to be fibroblasts, although in some cultures they were flattened and resembled epithelium. Accurate chromosome counts were made from drawings of 50 well spread metaphase plates. Results are presented in table 4 and figure 1.

TABLE 1

Distribution of chromosome numbers and of the numbers of cells with V-shaped elements. Lung of the lamb fixed 8 days after explantation.

Chromosome numbers		46	47	48	49	50	51	52	53	54	55
Number of cells	Total	1	1	..	3	1	1	3	3	35	2
	5 V	..	1	..	1	1	..	..	..	2	..
	6 V	1	..	..	2	..	1	3	3	33	2

Chromosome numbers were in the range of 46 to 55 with a modal value of 54 (70 per cent) and in a series of 33 out of 35 cells of this type, there were 6 large V-shaped chromosomes.

Microscopical examination was made of the individual chromosomes to identify homologous pairs as accurately as possible. Each chromosome was drawn with the aid of a camera lucida with special attention being given to size and shape (Figs. 2, 3). The drawings were then photographed and from prints individual chromosomes were cut out with the aid of a pair of scissors. These were placed in serial alignment according to size and the locus of the centromere. Two such ideograms are shown in figures 6 and 7. The serial alignment of the chromosomes as given in these figures indicates that there are 26 pairs of autosomes and the sex pair which consists of unequal X and Y elements. The first three pairs of autosomes are especially long, being V-shaped with metacentric centromeres. Among the fourth to twenty-sixth pairs there are at least 20 to 22 which are of the J-shaped type. These apparently have subterminal centromeres showing a tiny globular segment at one end, while the remainder are rod-shaped. The sex chromosomes are represented by a long J-shaped element, the X, and an extremely small grain-like body, the Y. The X element seems to have a length lying approximately between that of the sixth and seventh autosome.

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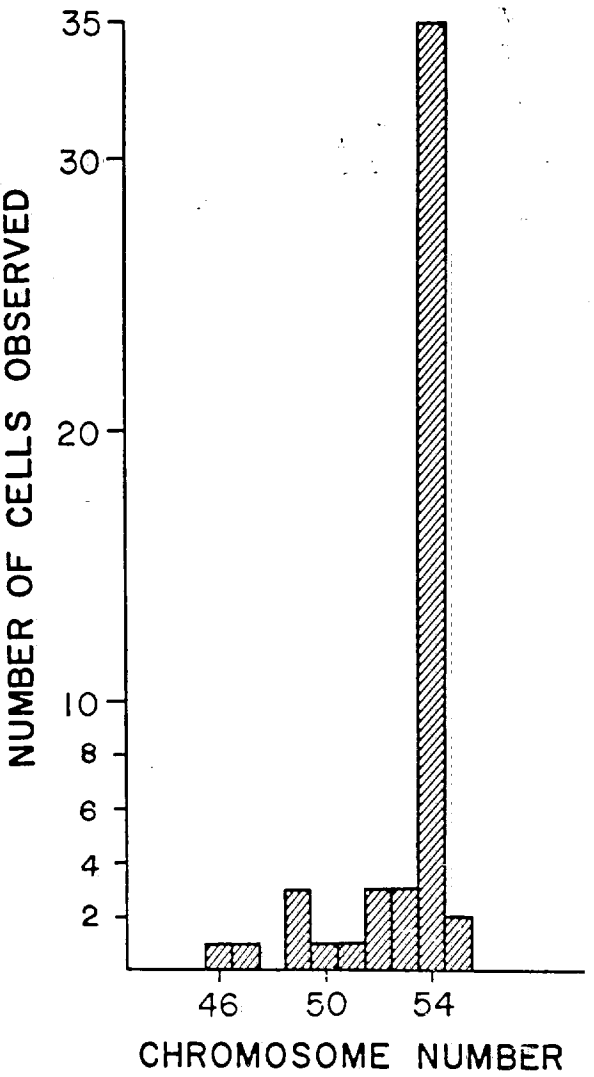

FIGURES 1 to 7 were based on cells in cultures of lung cells from a male lamb treated with colchicine on the 7th day and fixed on the 8th day of incubation.

FIG. 1. Distribution of chromosome number in lamb lung tissue. A total of 50 well spread figures were observed.

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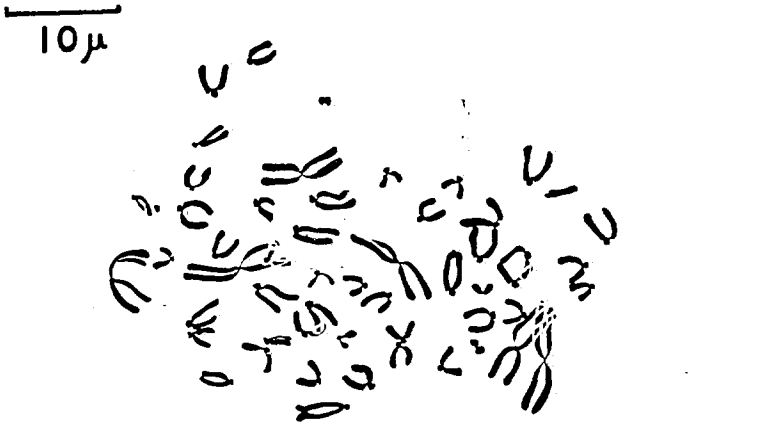

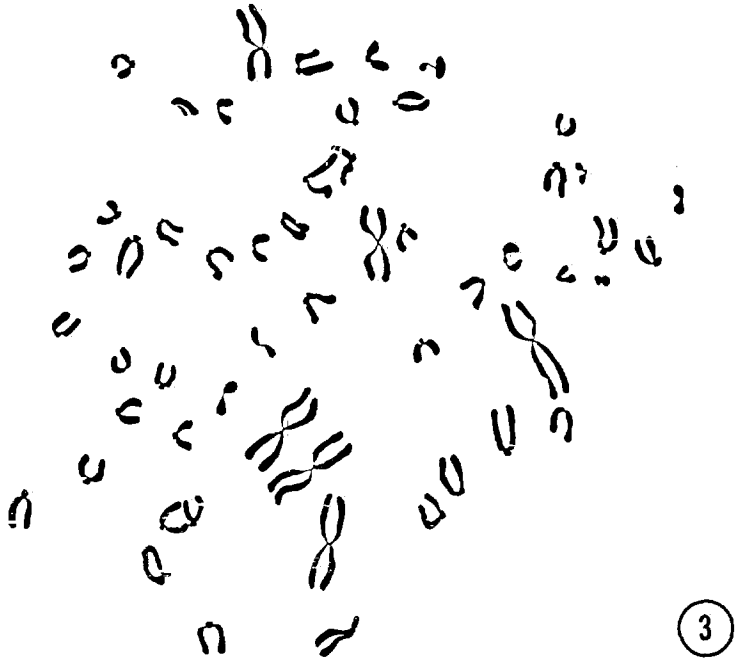

FIGS. 2 and 3. Camera lucida drawings of somatic chromosomes. The magnification for both drawings is given by the scale shown in figure 2.

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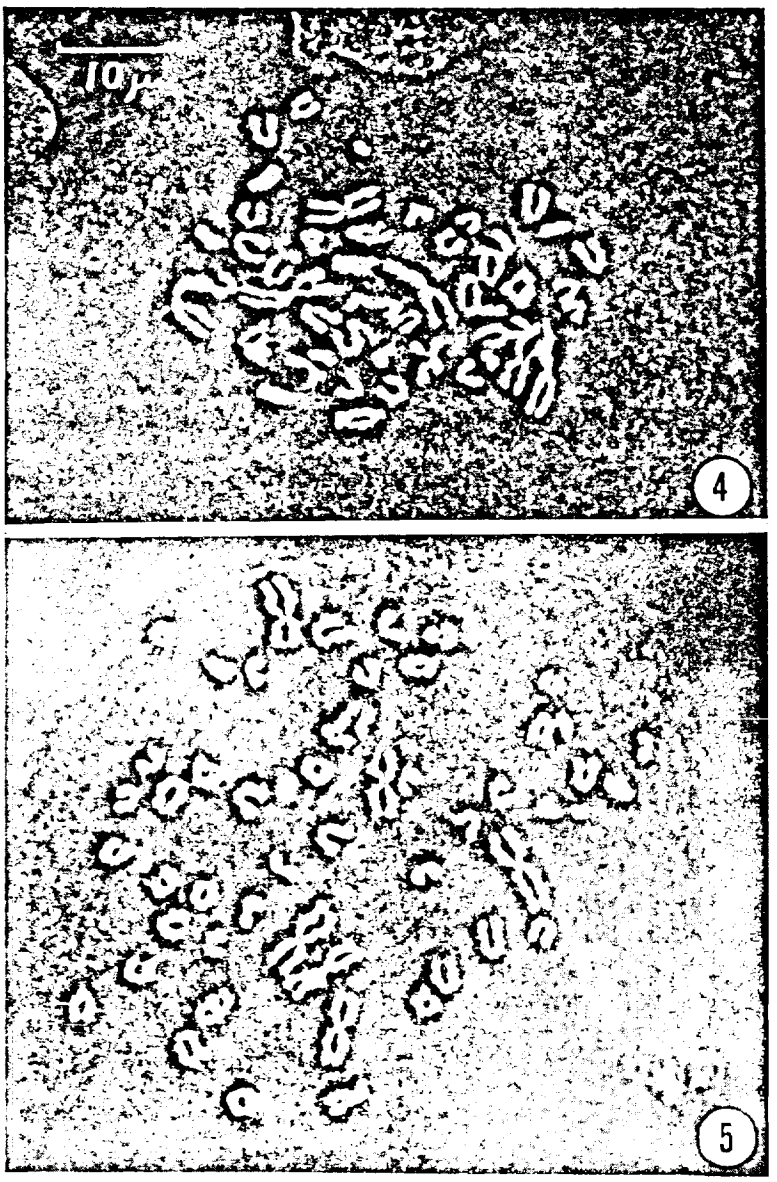

FIGS. 4 and 5. Photomicrographs of somatic chromosome. Figures 4 and 5 correspond to figures 2 and 3, respectively. The magnification for both photomicrographs is given by the scale shown in figure 4.

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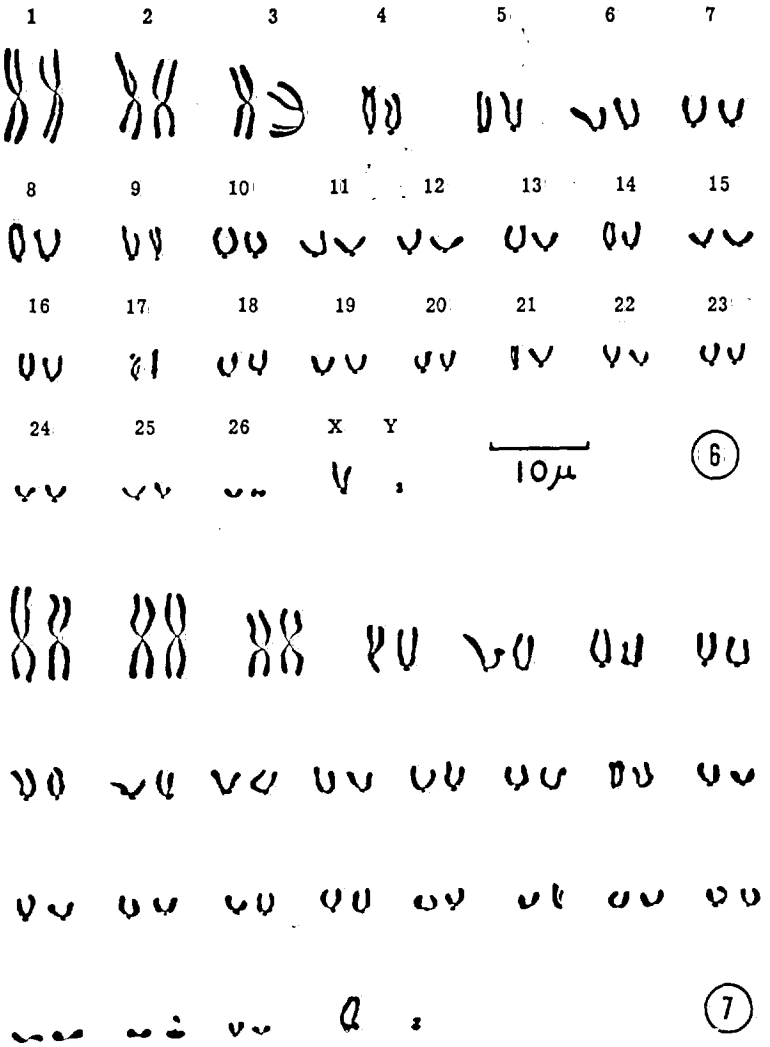

FIGS. 6 and 7. Serial alignments of chromosomes of the lamb in pairs which are presumed to be homologous in approximate order of size. Figures 6 and 7 correspond to figures 4 and 5, respectively. The magnification for both ideograms is given by the scale shown in figure 6.

Discussion

With regard to the chromosome number and in relation to some other morphological aspects, the result of the present study is in agreement, though not entirely, with the accounts given by

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Berry (1941) and Makino (1943). Ahmed (1940) reported the same number of chromosomes, but he stated that there were three large V-shaped chromosomes in the diploid set and that one of these was considered to be the X element.

Berry (1941) and Makino (1943) reported that the diploid complement of the sheep consisted of three homologous pairs of V-shaped chromosomes, 23 homologous rod-shaped pairs occurring in a gradual series and an unequal pair of the X and Y. The present observations, however, demonstrate that in the material obtained from the lung tissue of the lamb there are three pairs of V-shaped chromosomes and at least 20 to 22 pairs of elements having subterminal centromeres. The X element also carries this type centromere. The differences between the morphological data reported in this study and in those of previous reports (Berry 1941, Makino 1943) are obviously due mainly to the technical difficulties associated with the handling of cells containing large numbers of chromosomes. The development of the technique for the spreading of chromosomes in cell culture (Hsu and Pomerat, 1953) has made possible a more critical study of chromosome numbers and morphology.

For example, it had been thought on the basis of studies on germ cells that all rat chromosomes were rod-shaped elements having terminal centromeres until recent studies indicated that there are at least several pairs having subterminal and submedian centromeres according to the application of more modern techniques (Makino and Hsu, 1954; Ohno and Kinoshita, 1955; Tjio and Levan, 1956; Makino and Sasaki, 1958). As pointed out by Ohno and Kinoshita (1955) and Makino and Sasaki (1958), it seems to be possible, with further technical improvements, to establish that almost all elements of telomitic sets in various animal chromosomes are of acrocentric nature.

In the course of studies on the effect of tobacco condensates on human lung cells *in vitro*, the authors found shifts in the modal value and the development of dicentric chromosomes (unpublished). For such investigations the chromosome complex of lamb cells, even though involving large numbers, might prove useful in describing changes not only in chromosome counts but also in their shape because the peculiar combination of 3 long characteristic V-, 20–22 small J- and 1–3 rod shaped pairs might be traced more accurately.

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Summary

The somatic chromosomes of lung cells from a male lamb were studied in 8-day primary tissue culture preparations, since it is well established that the range of aneuploidy increases in the course of *in vitro* life. The most frequent chromosome number was determined as 54 (35 out of 50 cells). Morphological analysis revealed that the diploid complement of the lamb consists of 6 large V-, 40 to 44 J- and 2 to 6 rod-shaped autosomes. The X chromosome is J-shaped and the Y element is of the rod type.

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